Final Report

Tracking the source of *E. coli* contamination in Marquette Park Beach by DNA typing

Submitted by

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Objective

The primary objective of this study was to determine the impact of Gary sewage on the *E. coli* contamination of Marqutte Park Beach by comparing the DNA fingerprints of *E. coli* from the beach water with those from the Gary sewage. The secondary objective was to identify the possible sources of *E. coli* in the beach water using the existing Purdue University Calumet random amplified polymorphic DNA (RAPD) fingerprints database.

Materials and Methods

Sampling

Lake water samples were taken from three selected sites (A - Lake Street, B - Marquette Park, and C - Wells Street) of Marquette Park Beach on two dry (low risk) days and two wet (high-risk) days. A dry day is defined as no rainfall during the 72 h period prior to sampling, and a wet day is defined as heaving a rainfall and a combined sewer overflow event within 24 h prior to sampling. The first dry and wet day samples were collected in September 2001. The second dry and wet day samples were collected in July and August 2002, respectively.

Raw sewage samples were collected from the Gary sewage treatment facility

Enumeration of *E. coli* in Beach Water Samples

E. coli counts of the water samples were determined by the membrane filtration method using a Coliscan® Kit (Micrology Laboratories, Goshen, IN).

Isolation and Identification of *E. coli*

E. coli from beach water was isolated from Coliscan® plates. *E. coli* from sewage was isolated by spread-plating diluted raw sewage samples on MacConkey plates and incubated at 37 °C for 24 h. Randomly selected presumptive *E. coli* colonies from Coliscan® plates or MacConkey plates were dot inoculated on violet red bile-MUG (4-methylumbellifery-β-D-glucuronide) plates and incubated at 37°C for 24 h. All MUG (+) isolates were re-streaked on MacConkey plates and incubated at 37°C for 24 h. A single colony from each plate was confirmed and identified using BBL crystal ID Rapid Stool Enteric Kit (Becton Dickinson, Cockeysville, MD). Each confirmed *E. coli* isolate was kept on a tryptic soy agar slant (Difco) at 4°C and preserved in a Microbank (Pro-Lab, Ontario, Canada) vial at –80°C.

RAPD

A total of 254 confirmed *E. coli* isolates (204 from water and 50 from sewage) were used for RAPD analysis. The DNA of each was isolated using the genomic Prep™ DNA Isolation kit (Amersham, Piscataway, NJ). RAPD Analysis Beads (READY-To-Go™) (Amersham) were used for the RAPD reaction. A 5 µl solution containing 25 pmol of a primer was used per RAPD reaction. To the RAPD Analysis Beads in each reaction tube, the following were added: 5 µl solution containing 25 pmol of a single RAPD primer, 1 µl solution containing 10 ng of the

template DNA, and 19 µl of distilled water. PCR was performed with the Thermocycler 9700 (Perkin Elmer, Norwalk, CT) using the following cycles: 1 cycle at 95°C for 5 min followed by 45 cycles at 95°C for 1 min, 36°C for 1 min, and 72°C for 2 min. *E. coli* B was used as a positive control in all the RAPD reactions.

Gel Electrophoresis

To 4 µl of the amplified sample, 3 µl of 6X tracking buffer (30% glycerol, 0.125% bromophenol blue, 20 mM Tris-HCl, pH 8.0) was added. The sample mix was then loaded on a 2% agarose gel prepared with 1X TAE buffer containing 0.5 µg/ml of ethedium bromide. Electrophoresis was performed using HE 99X Super Submarine Unit (Amersham) at 150 volts for 3 h. The 100 bp DNA ladder (Amersham) was used as a sized marker in all gels.

Gel Analysis

DNA band patterns of all isolates were documented by the ImageMaster VDS system (Amersham). DNA fingerprints generated from 3 primers (Primer 2, 1247 and 1283) were combined and analyzed with the BioNumerics™ software (Applied Maths, Kortrijk, Belgium). Similarity between any two patterns was calculated using Pearson correlation which is based on densitometric curves. The optimization values were set at 1.25%, 1.50% and 2.00% for analyzing DNA patterns generated by primer 2, 1247, and 1283 respectively. Two RAPD patterns with 70% or more similarity are considered a close match.

Results and Discussion

E. coli counts of the water samples collected from 3 sites of Marquette Park Beach on each day are listed in Table 1. The water sample collected from site A consistently yielded higher *E. coli* counts than those of site B or C. Although the average *E. coli* counts on wet days were slightly higher than those on dry days, all the *E. coli* counts were far less than 235 CFU/ml, which is used as a standard for beach closure. According to the *E. coli* counts, the beach water samples were considered safe for human contacts on those 4 sampling days.

Table 1. *E. coli* counts of water samples collected from 3 sites on Marquette Park Beach on 2 dry and 2 wet days.

	Mean *E. coli* counts (CFU/ 100 ml)			
Sampling Day	Site A	Site B	Site C	Mean
Dry Day 1 (9/6/01)	26	10	13	16
Dry Day 2 (7/3/02)	45	26	11	27
Wet Day 1 (9/24/01)	55	27	35	39
Wet Day 2 (8/23/02)	96	14	14	41

RAPD patterns of beach water *E. coli* isolates were compared with those of Gary sewage *E. coli* as well as with all the RAPD patterns in the PUC database. The numbers of water isolates that matched the patterns in the PUC database or Gary sewage isolates are shown in

Table 2. On dry days, 20~26% of water isolates resembled those from human (HM) sources, and 18 to 32 % closely matched non-human (NHM) *E. coli*. On wet days, only 11 to 14% of the water isolates were identified as HM *E. coli*, while 52 ~ 63% were identified as NHM *E. coli*. There were only 4 out of 204 water isolates, 3 from day days and 1 from a wet day, matched the RAPD patterns of *E. coli* isolated from Gary sewage. Gary sewage was apparently not a major source of *E. coli* contamination in Marquette Beach water. A total of 58 water isolates (28%) yielded RAPD patterns that did not match any patterns in the database (Figure 1). Therefore, the sources of these *E. coli* isolates could not be identified.

Table 2. Identification of the possible sources of *E. coli* isolated from Marquette Park Beach water samples by matching the *E. coli* RAPD patterns with those in the database.

Possible	Dry Day		Wet Day	
Sources	#1	#2	#1	#2
HM				
Fecal or Urine (245*)	6/50 (12%)	7/50 (14%)	5/54 (9%)	7/50 (14%)
Gary Sewage (50)	2/50 (4%)	1/50 (2%)	1/54 (2%)	0/50 (0%)
Other Sewage (44)	2/50 (4%)	5/50 (10%)	0/54 (0%)	0/50 (0%)
Total	10/50 (20%)	13/50 (26%)	6/54 (11%)	7/50 (14%)
NHM				
Seagull (86)	12/50 (24%)	5/50 (10%)	14/50 (26%)	14/50 (28%)
Goose (55)	1/50 (2%)	5/50 (10%)	1/54 (2%)	5/50 (10%)
Farm Animals (140)	13/50 (26%)	5/50 (10%)	7/54 (13%)	7/50 (14%)
Raccoon, Deer (100)	6/50 (12%)	3/50 (6%)	12/54 (22%)	0/50 (0%)
Total	32/50 (64%)	18/50 (36%)	34/54 (63%)	26/50 (52%)
Unknown (No match)	8/50 (16%)	19/50 (38%)	14/50(28%)	17/50 (34%)

*The number of RAPD patterns in the database.

Figure 2 shows the matching results of the possible sources of *E. coli* on dry and wet days. On wet days, the percentage of NHM *E. coli* increased and the percentage of HM *E. coli* decreased when compared with the dry day results. This data suggests that the land run off could wash animal fecal materials into the lake and increased NHM *E. coli* in the water on wet days. Based on the low *E. coli* counts (Table 1) and low percentage of HM *E. coli* contamination in wet day samples (Figure 2), it appears that the CSO events occurred before the two wet sampling days had very little influence on the *E. coli* contamination of Marquette Park Beach.

Among the NHM sources, seagulls were an important source of *E. coli* in water. Ten to 28% of the isolates were identified as seagull *E. coli* (Table 2).

Conclusions

1. According to the current DNA fingerprint data, no evidence suggested that Gary sewage or other sewage had a significant impact on the *E. coli* contamination of Marquette beach on the four sampling days.

2. Overall, RAPD patterns of less than 20% of beach water *E. coli* isolates resembled those of HM *E. coli*, while more than 50% matched to NHM *E. coli*.

3. On wet days, rain washed animal fecal materials to the lake and thus further increased the percentage of NHM *E. coli* in water.

4. Among NHM sources, seagulls seemed to be important contributors of *E. coli* in water.

5. The CSO events prior to the 2 wet sampling days had little influence on the *E. coli* contamination of Marquette Park Beach.

Figure. 1. Possible sources of *E. coli* in all 4 Marquette Park Beach water samples.

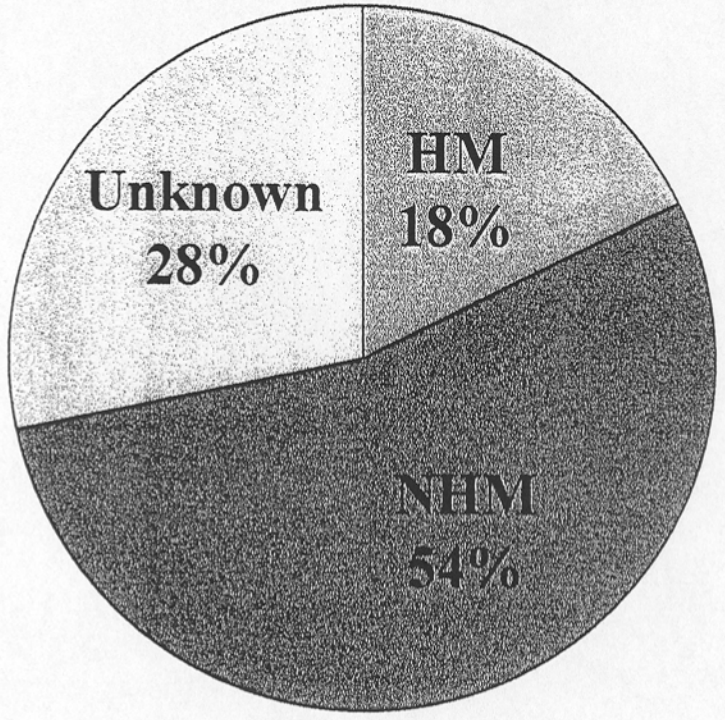

Figure. 2. Comparison of sources of *E. coli* in Marquette Park Beach water samples collected from 2 dry and 2 wet days.